Arch. Bas. App. Med. 7 (2019) 7 – 11
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ARCHIVES OF BASIC & APPLIED MEDICINE

Research Article

Comparative Studies of the Effects of Ripe and Unripe Peels of *Musa paradisiaca* and Sofratulle® on Excised Wound Healing in Rat

Ajani R.S. and Oguntokun O.M.
Department of Anatomy, College of Medicine, University of Ibadan.

Received: October 2018; Revised version accepted: January, 2019

Abstract
Musa paradisiaca (plantain) is widely cultivated for nutritional purposes with many health values being attributed to it. Its peel, which constitutes a significant proportion of the plant and usually disposed off as biological waste, has been reported to be effective in wound repair processes. In this study, the effects of ripe and unripe peels of powder and ethanolic extracts of *Musa paradisiaca* on wound healing were compared with softratulle, a standard wound-dressing agent. The excisional wounds of thirty-six adult male Wistar rats in six equal groups were dressed with normal saline (NS), sofratulle (SF), ripe peel powder (RPP), unripe peel powder (UPP), ripe peel ethanolic extract (RPE) and unripe peel ethanolic extract (UPE) daily till healed. Mean wound contraction rates were determined from wound sizes every three days. Granulation tissue taken from an animal in each group every three days and together with end scar tissues were processed for histological analyses. The mean wound contraction rate on day 3 for the UPP group was significantly higher that of the RPP group. Also on day 3, that of the UPE was similarly significantly higher than that of RPE. Although, the mean wound contraction rates of the control (NS and SF) were significantly higher than those of the peel groups on days 3,6 and 9, by day 12, the differences became insignificant. Histology of the granulation and scar tissues of both control and experimental showed similar features consistent with wound healing. Thus the powder and ethanolic extracts of ripe and unripe peel of *M. paradisiaca* accelerated healing of excisional wounds of Wistar rats.
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Key Words: *Musa paradisiaca , wound healing, ripe and unripe peel, sofratulle*

INTRODUCTION

Wound is a breach in the integrity of the skin having diverse causes. Irrespective of its aetiology and provided the affected part of the body is still viable, the restorative process known as wound healing sets in. Wound healing is a natural phenomenon that may be accelerated or retarded by intrinsic or extrinsic factors. The roles of these factors necessitate intervention in form of wound dressing. Such dressing materials include honey (Jull *et al*.,2008), collagenase (Shi *et al*.,2010), maggots (Sherman,2009; Marineau *et al*.,2011; Shi and Shofler, 2014; Arabloo *et al*., 2014), and Eusol (Humzah *et al*., 1996). Although, there are main synthetic mateials available for the management of chronic wound; one of the main problem is their cost effectiveness (Von Atzingen *et al*.,2013). Unripe banana peel is known to contain a flavonoids, antioxidants and beta carotene that induce cell proliferation thus accelerating wound healing (Lewis *et al*.,1999; Someya *et al*.,2002).

Plantain (*Musa paradisiaca*) is a perennial plant that has fleshy edible fruit with thick skin known as peel. The peel constitutes about 40% of the total weight (Eun-Hye etal.,2010) of fresh plantain and usually disposed off as waste or fed to ruminants. *Musa paradisiaca* is widely cultivated and consumed in Nigeria thus a very large amount of its peel is generated which contributes significantly to organic waste.

Waste management is a big environmental issue in most African countries including Nigeria, thus any measure at making waste to be useful will be of tremendous benefit and relief. Isolates of the peel of *Musa paradisiaca* include cellulose and amino acids such as arginine, leucine, valine and aspartic acid (Kekitu,1973; Emaga *et al*.,2007). Also the peel of M. paradisiaca has been found contain antioxidants which are capable of accelerating wound healing (Padill-Carberos et.al.,2016). Amino acids are essential for neovascularization and collagen synthesis; these are part of the stages of wound healing. Finding the peel of M. paradisiaca being beneficial to wound healing will thus reduce the burden of waste management hence the justification of this study.

MATERIALS AND METHODS

Preparation of plant material

Ripe and Unripe matured *Musa paradisiaca* were procured from a commodity market in Ibadan, south west Nigeria. The fleshy edible portions were completely removed. The skin i.e the peel which is normally discarded was used for the study. The peels were chopped into bits, dried at room air and subsequently milled into powder.

Ethanolic extracts: Three hundred and ninety grammes (390g) of RPP and 530 g of UPP were used for the ethanolic extractions giving a yield of 10 % and 14 % respectively. Each

**Author for Correspondence:* Tel: +234-802-300-7362 E-mail: rsaajani@yahoo.co.uk

of the extracts (ripe peel ethanolic extract{RPE}; unripe peel ethanolic extract {UPE}) was separately mixed with propylene glycol to obtain a gel that was subsequently used as dressing material.

Crude powder: A gel of the ripe peel crude powder (RPP) was obtained by mixing 435 g of the powder with propylene glycol. While 592 g of the unripe peel powder was used to prepare its gel (unripe peel crude powder{UPP}).

Phytochemical analysis

Three hundred and fifty grammes each of the ripe peel powder (RPP) and unripe peel powder (UPP) were used for qualitative analyses while 15 grammes of each of RPP and UPP were used for quantitative analyses.

Animals

Thirty-six adult male Wistar rats weighing 150-250 g were used for the study. They had an initial two weeks of preconditioning under controlled environmental temperature, humidity and adequate aeration and illumination were ensured. They had pelletized feed and water.

Design of the Experiment

The determinant for group allotment was the dressing material thus six groups of six animals each were created. They were Normal saline (NS), Sofratulle (SF), Ripe peel powder (RPP), Unripe peel powder (UPP), Ripe peel ethanolic extract (RPE) and Unripe peel ethanolic extract (UPE).

Wound Creation and Management

Wound creation and management were as previously described by Ajani *et al* (Zamide *et al*., 2015; Ajani and Ogunbiyi, 2015).

Each rat was sedated with intramuscular ketamine hydrochloride (120 mg/kg). After initial cleansing with savlon antiseptic liquid, a 2 cm by2 cm full thickness skin about 1.5 cm from the vertebral column was excised.

Wound Management and Data Collection

Normal saline, Sofratulle, ripe peel crude powder, unripe peel crude powder, ripe peel ethanolic extract and unripe peel ethanolic extract was used as wound dressing material for the respective group (NS, SF, RPP, UPP, RPE and UPE). The wounds were dressed daily till healed. Prior to change of dressing, wound size estimation was done by taking measurements along two perpendicular planes at an interval of three days. From the obtained values, wound contraction rates were calculated in percentages. Granulation tissue was harvested from a member of each group on day 3, 6 and 9. These samples were processed for light microscopy using Heamatoxylin and Eosin (H&E) stain. The processed granulation tissues were used for evaluation of wound healing in the groups in terms of cellularity, angiogenesis, fibroplasia and collagen synthesis. Animals that had granulation tissue excised on these days were removed from the study. The wounds of the other members of the groups were allowed to heal and the scars were in similar fashion processed for light microscopy.

Data Analysis and Processing

The numerical aspects of the results were analyzed with Statistical Package for the Social Sciences (SPSS) version 21 and expressed as percentages, means plus standard deviation of

means (SD). The student t- test was used for inter group comparison and level of significance

was set at $p<0.05$.

RESULTS

Phytochemical analyses

The phytochemical types and contents of both ripe and unripe *Musa paradisiaca* were essentially the same. Anthrquinones and terpenoids were present in significant quantities while alkaloids (0.06%), flavonoids (2.8-5.56%) and taninis were of small quantities. Both saponins and cardiac glycosides were not detected (Table 1).

The normal saline (NS) group had the highest mean wound contraction rate of 39.29±12.13 % on day 3 while the ripe peel ethanolic extract (RPE) and ripe peel powder (RPP) groups had the least (9.88±2.09,8.99±2.82 % respectively). Though both the RPE and RPP still had the lowest mean wound contraction rates at day 6, it is worthy to note that both had increased by up to 200 % to 23.88±6.09 and 20.44±2.93 % respectively. By day 9, the mean wound contraction rate in the NS group had a marginal increase from 58.29±6.40 to 65.31±4.30 % while the Sofratulle (SF) group had the highest rate of 80.42±3.57. The lowest mean wound contraction rate for day 9 was obtained from the ripe peel powder (RPP) group. The remaining groups RPE, unripe peel ethanolic extract (UPE) and unripe peel powder (UPP) had similar rates for the same period. (Table 2). By day 12, the NS, SF and RPP had achieved over 80 % mean wound contraction rates with that of the UPE being the least (71.96±7.91 %). Very reasonable degree of healing had occurred in all groups as at day 15 as evidenced by more than 80 % mean wound contraction rates (Table 2).

Comparisons on mean wound contraction rates between the groups

On day 3 of the experiment, the mean contraction rate of the NS group was significantly higher than those of all the experimental groups whereas; the SF group had significantly higher rate than those of the RPE, UPE and RPP groups over the same period. Only the NS group had a mean wound contraction rate that was significantly higher than those of the experimental groups on day 6. On day 9, the difference of the mean wound contraction rate between the NS group and the respective experimental group RPE, RPP and UPP was statistically significant. While all the experimental groups had statistically lower mean wound contraction rates with reference to that of the SF group.

Table 1:
Phytochemical analyses of ripe and unripe *Musa paradisiaca*

TEST	Anthraquinones	Terpenoids	Alkaloids	Flavinoids	Tanins	Saponins	Cardiac Glycosides
Qualitative	++	+++	+	+	+	-ve	-ve
Quantitative			0.06 %	2.8-5.56 %			

Table 2:
Interval mean wound contraction rates in percentages (%)

Group	Day 3	Day 6	Day 9	Day12	Day 15	Day 18	Day 21	Day 24
NS	39.29±12.13	58.29±6.40	65.31±4.30	92.38±3.22	94.06±3.56	94.67±2.50	96.20±2.20	Healed
SF	24.23±9.91	35.05±7.15	80.42±3.57	86.64±3.19	92.04± 3.84	Healed	Healed	Healed
UPP	21.67±11.13	22.36±10.81	42.00±14.37	78.59±6.71	81.82±1.78	92.92±0.86	Healed	Healed
RPP	8.99±2.82	20.44±2.93	39.95±8.71	80.95±6.43	85.50±0.02	90.84±0.00	92.37±0.00	Healed
UPE	14.70±5.85*	25.63±11.04*	42.00±14.37	71.96±7.91	81.27±0.51	88.42±2.91	92.05±5.47	Healed
RPE	9.88±2.09*	23.88±6.09*	42.21±12.40*	75.92±9.55	91.75±6.01	96.09±2.71	Healed	Healed

NS- Normal saline, **S**F-Sofratulle, RPE- Ripe peel ethanolic extract, UPE- Unripe peel ethanolic extract, RPP- Ripe peel powder, UPP- Unripe peel powder.
* P <0.05 vs NS for the same day

The mean wound contraction rate of the NS group was observed to be significantly higher than that of the SF group over the same period i.e. day 3, 6 and 9. It should be noted the differences in this rate between the control groups (NS and SF) and the experimental groups from day 12 onwards were insignificant (Table 3).Comparing these rates graphically, all the experimental groups had greater upward gradients than the SF and NS groups between day 3 and day 12. However, all the groups (experimental and control) flattened out from day 12 upwards.

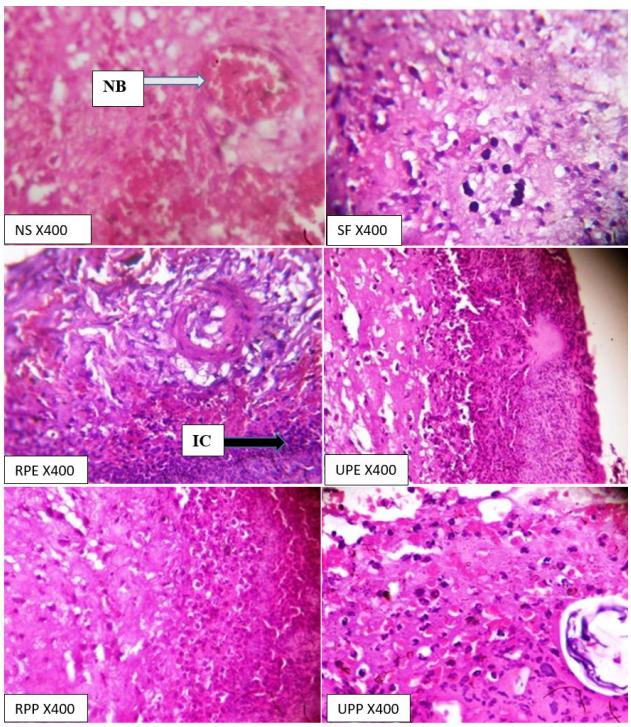

Plate 1
Granulation tissue at Day 3 H& E
NS (Normal saline), SF (Sofratulle), RPE (Ripe peel ethanolic extract), UPE (Ripe peel ethanolic extract), RPP (Ripe peel powder) and UPP (Unripe peel powder). NBV (New blood vessel), IC (Inflammatory cells).

Granulation tissues and wound scars
Microscopy of the granulation tissues on day 3 revealed presence of inflammatory cells and fibroblasts in all the groups; however, the RPE and UPE groups had the largest cellular density (Plate 1) Also new blood vessels (evidence of angiogenesis) could be seen. By day 6, the granulation tissues in NS, SF, RPE, UPE and RPP showed increased cellularity with fibroblasts predominance (Plate 2). By 9, the granulation tissues of the peel groups displayed more of collagen fibres and less of fibroblasts whereas, fibroblasts were more obvious in the SF group (Plate 3). Histology of the sections prepared from the scars showed predominance of fibrillary structures, less cellularity with well-defined epidermal and dermal layers. These features were of same magnitude in the control (NS & SF) peel extracts (ripe and unripe) but of reduced magnitude in the peel powder (RPP and UPP) (Plate 4).

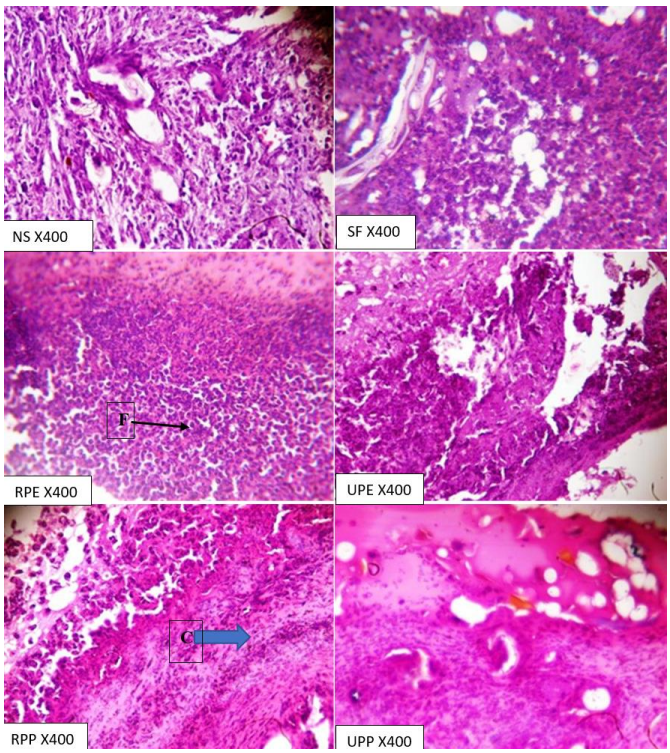

Plate 2.
Granulation tissue at Day 6 H& E
NS (Normal saline), SF (Sofratulle), RPE (Ripe peel ethanolic extract), UPE (Ripe peel ethanolic extract), RPP (Ripe peel powder) and UPP (Unripe peel powder). F (Fibroblast), C (Collagen)

DISCUSSION

In wound healing, there is a systematic, programmed replacement of the devitalized tissue with fibrous tissue. Also wound healing is the process by which the morphology of the damaged tissue is restored. Its phases are hemostasis, inflammation, proliferative and remodeling.

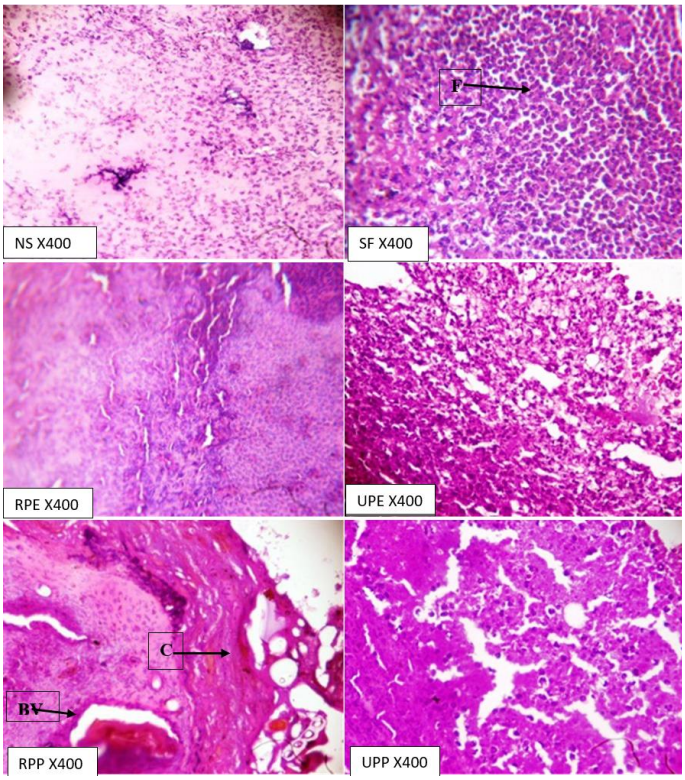

Plate 3.
Granulation tissue at Day 9 H& E
NS (Normal saline), SF (Sofratulle), RPE (Ripe peel ethanolic extract), UPE (Ripe peel ethanolic extract), RPP (Ripe peel powder) and UPP (Unripe peel powder) C (Collagen), F (Fibroblast), BV (Blood vessel).

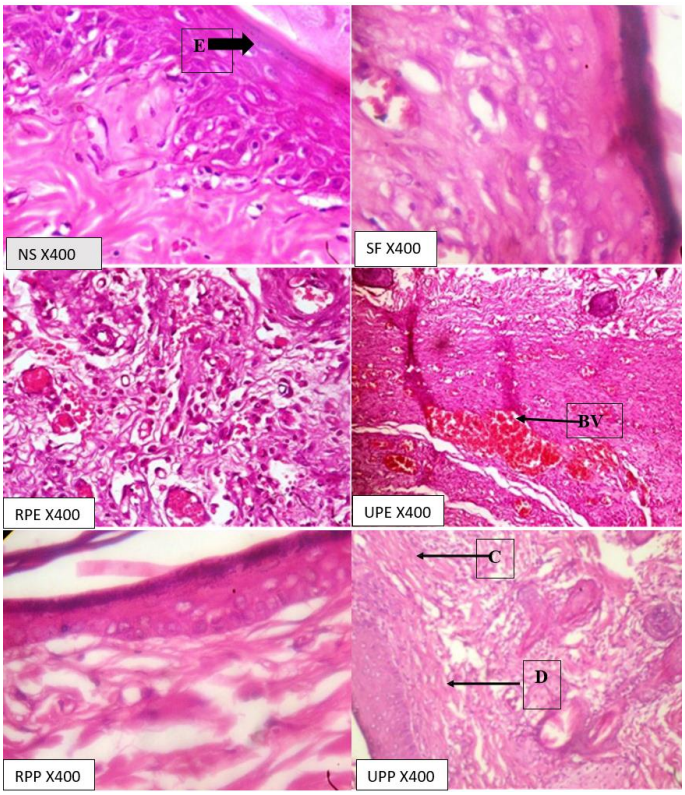

Plate 4:
Sections from the wound scars
NS (Normal saline), SF (Sofratulle), RPE (Ripe peel ethanolic extract), UPE (Ripe peel ethanolic extract), RPP (Ripe peel powder) and UPP (Unripe peel powder), E (Epidermis),D (Dermis) C (Collagen), BV (Blood vessel).

The major component of the remodeling phase is wound contraction and it entails progressive reduction in the size of the wound thus its rate is a very reliable parameter in the assessment of the progression of healing. The mean wound contraction rate on day 3 for the unripe peel powder (UPP) group was significantly higher that of the ripe powder (RPP) group. Also, on day 3, that of the unripe ethanolic extract (UPE) was similarly significantly higher than that of its ripe counterpart (RPE). However, for the subsequent intervals of the study the differences were insignificant thus being ripe or unripe, did not confer any advantage on plantain peel as a wound dressing material in Wistar rats. Comparisons of the mean wound contraction rates between the powdery and ethanolic extract of the peel (ripe and unripe) did not reveal any significant difference, thus using *M paradisiaca* either in crude powder form or its ethanolic extract (both ripe and unripe) are of equal potency as wound dressing materials in rat. This should be of paramount importance since it was easier and simpler to prepare the powdery form than the ethanolic extract. This may be of translational importance to its application in the management of human wounds.

Comparison of the mean wound contraction rates between normal saline (NS) and the experimental groups (RPP, UPP, RPE and UPE) showed that NS had significantly higher rate than each of these groups on days 3,6 and 9 however, by day 12, the differences became insignificant. This observation could suggest that both the powder and ethanolic extracts of ripe and unripe *M paradisiaca* have wound healing potentials that could compare favourably with that of normal saline though with an initial lag. Also, comparisons of the mean wound contraction rates between sofratulle (SF) the experimental groups showed that the SF group had significantly higher rates on days 3 and 9 but not on day 6. A closer consideration of the interval mean wound contraction rates revealed that rate for SF increased by about 50 % from day 3 to day 6, whereas by 9 it increased by more than 100 %. This might explain the pattern of comparison outcome between the SF group and the experimental groups. It should be noted that the wounds of the animals in the SF group were the first to heal also the mean contraction rates of the SF group were significantly higher than those of the NS group on days 3,6 & 9. This might be due to the antibiotic (framacytin) component of sofratulle. Although, the wounds were freshly created, they were of the clean contaminated category but not infected. The antibiotic minimized the inflammatory response thus shortening the inflammatory phase leading to early synthesis of myofibrils by fibroblasts. The myofibrils are normally responsible for wound contraction (Junqueira and Carneiro, 2005). Among the peel groups, the ripe peel ethanolic extract (RPE) was the first to achieve epithelization i.e. healing, this compared favorably with SF group thus it might be inferred that ethanolic extract of *M. paradisiaca* promoted wound healing to an extent similar to that of sofratulle.

Comparison of the mean wound contraction rates revealed that all the experimental groups had greater upward gradients than the SF and NS groups between days 3 and 12. However, all the groups (experimental and control) flattened out from day 12 upwards. The inference from this observation was that both dry powder and ethanolic extract of ripe and unripe plantain peel have wound healing potentials comparable with those of Sofratulle and normal saline though with initial lagging when the wound contraction rate is the parameter.

Thus, in the absence of other morbidities such as sepsis, compromised renal functions; plantain peel can be used in substitution for Sofratulle as wound dressing material.

The phytochemical analyses of the ripe and unripe *M. paradisiaca* used in this study were similar to those of relevant studies (Ighodaro,2012; Dei *et al*.,2007). Such phytochemicals that include anthraquinones, terpenoids, alkaloids, flavonoids, tanins and saponins have been found to be free radical scavengers, antioxidants, antimicrobial and astringent (Dash and Murthy,2011; Savithramma *et al*.,2011; Soni and Singhai, 2012). These may thus be the mechanisms by which *M. paradisiaca* peels (ripe and unripe) accelerate wound healing. Serial sections of the granulation tissues in the peel groups were consistent with those of normal phases of wound healing and also with those of the control. Also, the histology of the scars of the experimental groups were similar to those of the control groups. Thus, from both quantitative (wound contraction rates) and qualitative (histology of the granulation and scar tissues) evidences; the peel of *M. paradisiaca* (powder and ethanolic extracts) promote wound healing in Wistar rats.

In conclusion, considering the fact that the peel of *M. paradisiaca* is said to constitute about 40% of its weight, finding a role for it in the management of wound would amount to value addition and also reduce biologic waste generation and improved waste management.

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